The Best of Both Worlds: Identification of more Proteins in Rat Liver by Semi-Automated 2D-PAGE-Nanoflow LC/MS/MS

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Overview

Purpose

- To combine the strengths of 2D PAGE and LC/MS/MS for the identification of proteins by using an automated gel-processing instrument, bioinformatic tools and a robust nanoflow LC/MS/MS procedure.

Methods

- Ninety-six randomly selected spots from a 2D PAGE separation of rat liver proteins were prepared for nanoflow LC/MS/MS analysis using the Xcise™ automated gel-processing instrument.
- Nanoflow LC/MS/MS analysis was performed on an LCQ ion-trap mass spectrometer equipped with a ProteCol™ capillary column in which the column, endfittings, frit, and tubing are integrated into a single unit.
- Data files from nanoflow LC/MS/MS experiments (.raw) and database searches (SEQUEST .dta and .out) were stored and managed using an integrated bioinformatic platform (BioinformatIQ™).

Results

- Nanoflow LC/MS/MS analysis of ninety-six 2D PAGE spots resulted in the identification of seventy-seven rat liver proteins, twenty-four of which have never previously been observed in proteomic studies of rat liver. [2-3]
- Using bioinformatic tools and the Xcise™ automated gel-processing instrument, sample-tracking information including gel spot location was preserved within BioinformatIQ™.

Introduction

2D PAGE is a protein separation technique able to resolve highly complex protein mixtures. Protein "spots" are excised from the gel, destained, digested, and the resulting peptides purificated prior to mass spectrometric analysis. MALDI-MS for protein identification can fail when multiple proteins are present. Proteins can also be identified using nanoflow LC/MS/MS which has a greater capacity to identify multiple proteins similtaneously.[1] Nanoflow LC/MS/MS combines a miniaturised liquid chromatographic separation with a tandem (MS/MS) mass spectrometer, providing separation, detection, and fragmentation of the peptides under investigation.

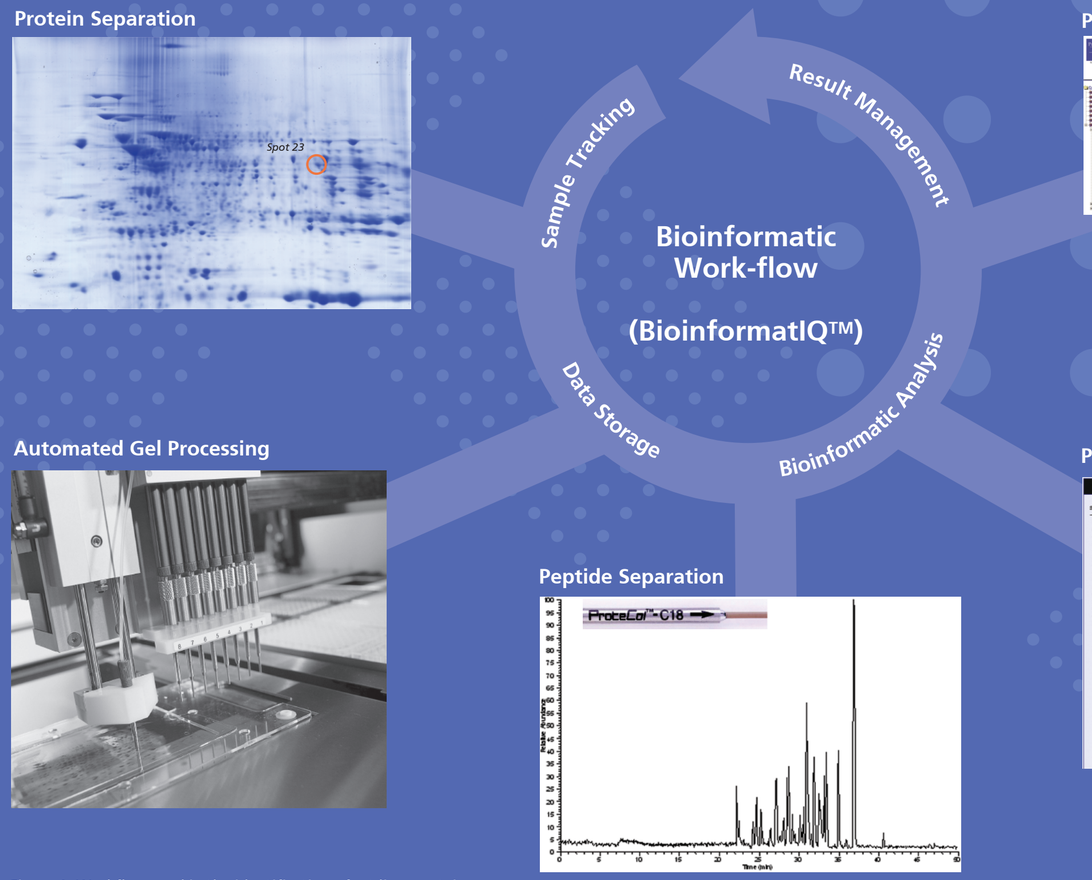

Figure 1. Workflow used in the identification of rat liver proteins.

Methods

Proteins from whole rat liver were separated by 2D-PAGE (pI 3-10 IPG and 6-15% GelChip) using an ElectrophoretIQ³™ instrument. A total of 1065 spots (commassie stained) were detected using ImagepIQ™. Ninety-six randomly selected spots were excised, destained, digested (trypsin) and prepared for nanoflow analysis using the Xcise™. Nanoflow LC/MS/MS was performed on an LCQ Deca ion-trap mass spectrometer. Peptides were trapped prior to separation on a reverse phase ProteCol™ column (SGE, C18, 3µm, 300Å, 150µm X 100mm). Autorun sequence files were generated using BioinformatIQ™. Bioinformatic tools (agent and daemon) were used to coordinate the acquisition of data on the MS workstation, including the transfer of data file (.raw) to a server in preparation for SEQUEST searches (Bioworks 3.1). MS/MS spectra were searched against a database containing all rat protein sequences in the Swiss-Prot/TrEMBL database. Proteins were assigned using a bioinformatic application (BioinformatIQ™) designed specifically for collecting data in large scale proteomic projects.

Results

Figure 1 illustrates the workflow used in the identification of rat liver proteins. The average sequence coverage and average number of peptides identified for proteins grouped into five different molecular weight ranges is displayed in Figure 2. Figure 3 illustrates the number of proteins identified per excised spot. Spot intensity values obtained using ImagepIQ™ were used to group spots into three categories (light, medium and dark spots). Figure 4 illustrates the average protein sequence coverage obtained using nanoflow LC/MS/MS for spots of light medium and dark intensity.

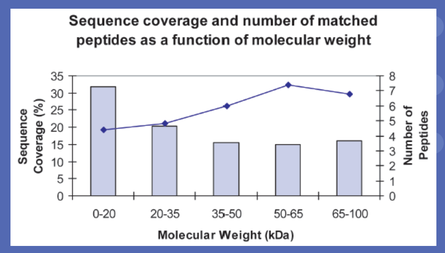

Figure 2. Sequence coverage and number of peptides identified for proteins grouped into five different molecular weight ranges.

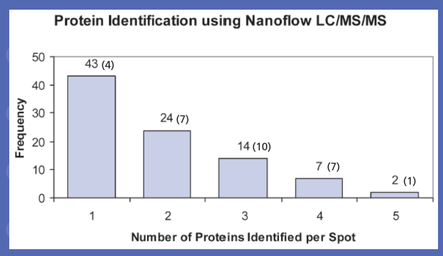

Figure 3. Number of rat liver proteins identified per 2D PAGE spot. Values in brackets indicate the number of spots that contain proteins never previously observed in studies of rat liver.

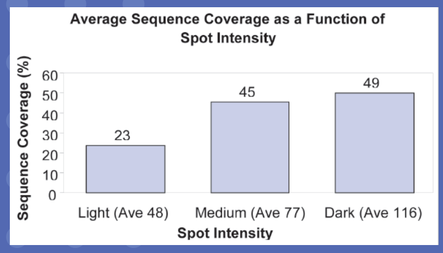

Figure 4. Average protein sequence coverage obtained for spots of light medium and dark intensity

Proteins were successfully identified for 94% of all spots analysed by 2D PAGE/nanoflow LC/MS/MS. In total, seventy-seven proteins were identified, many as isoforms, twenty-four of which have never previously been identified in proteomic studies of rat liver.[2-3] Almost fifty percent of the 2D separated spots analysed by nanoflow LC/MS/MS contained multiple proteins.

Conclusion

- Semi-automated 2D PAGE/LC/MS/MS combines the parallel purification of proteins and their isofoms, with the sensitive analytical capabilities of nanoflow LC/MS/MS.
- The identification of 24 proteins not previously observed in the rat proteome emphasises the power of this proteomics approach.

References

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